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TECHNICAL MANUSCRIPT 383

INHIBITION OF MITOSIS BY SURFACTANTS

Arthur A. Nethery

MARCH 1967

DEPARTMENT OF THE ARMY
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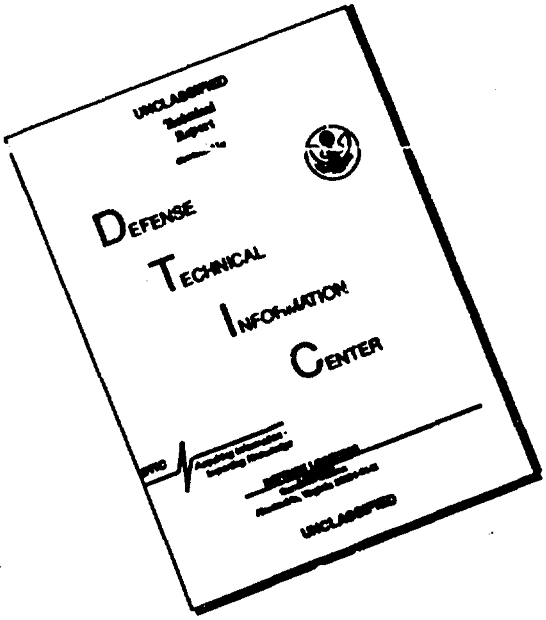

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INHIBITION OF MITOSIS BY SURFACTANTS

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Crops Division
BIOLOGICAL SCIENCES LABORATORY

Project 1C522301A061 March 1967

ABSTRACT

Twenty-two ionic and nonionic surface-active agents were applied to a standardized pea root meristem test system. Mitosis was inhibited by 16 surfactants at concentrations of 0.1% v/v. Two surfactants caused a slight depression in the mitotic index; the remaining four had no recognizable effect. Several compounds were irreversibly toxic at levels of 0.1%. Five of the six known biodegradable surfactants tested were toxic.

I. INTRODUCTION

The use of surface-active agents as tools in biological research has become a common practice. The usefulness of such agents depends largely on their ability to alter the energy relationships at interfaces. Certain surfactants exhibit independent effects on biological systems, including osmotic changes,[1] protein denaturation,[2] cytolytic injury,[3] and either enhancement or inhibition of growth.[4] Surfactants may be used to wet plant surfaces or suspend, disperse, or emulsify other chemical agents used in treating the plant materials. When the influence of such chemicals on cell division or growth is being studied, it is also important to determine the effects of the surfactants on these processes.

Nonionic surfactants should be chemically rather inert, because of their lack of ionization. Thus, they have been used more commonly than cationic, anionic, and amphoteric (ampholytic) surfactants. However, a number of nonionic surfactants have been reported to stimulate growth of plant parts,[5-7] to inhibit growth in plants,[6-8] and to enhance the effect of various herbicides.[4,9-14]

It is generally agreed[4,14,15] that the cationic surfactants are the most phytotoxic class. Because of the diversity of the responses of different plant species, the various modes of application, the range of surfactant chemical structures, and the spectrum of dose levels used, generalizations cannot be made from the published data regarding the relative effects on plants of the other classes of surfactants.

A series of experiments was carried out to determine the effects of a number of surfactants, including representatives of all four general classes, on mitosis in root meristems of pea seedlings.

II. MATERIALS AND METHODS

All tests were carried out on seedlings of Pisum sativum var. Alaska, according to the method described by Wilson.[16] The seeds were soaked 6 hours in distilled water, then rolled in paper toweling moistened with distilled water. The paper toweling rolls were maintained at 25 C and 50% relative humidity for 42 hours. At the end of this period, the seedlings were selected for uniformity of appearance (root length, 1½ to 2 cm), placed on wire mesh grids coated with acrylic plastic, suspended over 1-liter plastic pots containing aerated one-half strength Hoagland's nutrient solution, and allowed to acclimatize for 4 hours. The grids were then transferred to the treatment solutions for 4 hours. At the end of this period, they were returned to the original solution. Samples were taken at various intervals during the treatment period and for 24 hours after the treatment. Control samples were taken prior to the treatment, and appropriate untreated control samples were taken throughout the experiments.

Mitotic disruption was analyzed by determining the mitotic index of the pea root meristems at the end of the treatment period. The mitotic index is expressed as the number of dividing cells per 1,000 cells scored. The term toxicity as used here does not necessarily imply total plant toxicity, nor inhibition of secondary root growth subsequent to removal from treatment, but rather denotes an irreversible cessation of cell division and growth in the primary root. A surfactant was considered toxic at a given concentration if the mitotic index of the root meristem had not recovered to the control level 24 hours after treatment.

The trade names, descriptions of chemical structures, chemical types, and manufacturers or suppliers of all surfactants tested are shown in Table 1. All compounds were tested at a concentration of 0.1% v/v or 0.1% w/v as supplied. When the active ingredient was known to be less than 100%, the concentration of the treatment solution was adjusted to provide 0.1% active ingredient. This concentration was selected because it is equal to or greater than the critical micelle concentration (cmc) range of all compounds tested, with the possible exception of Peregal ST. The formation of micelles within a narrow concentration range is characteristic of most surfactants, and most deleterious effects on plants have been induced at concentrations above the cmc range.[12] The cmc ranges were estimated by a qualitative dye color change method, using fluorescein for cationic surfactants, Pinacyanol chloride for anionics, and benzopurpurine 4B plus HCl for nonionics. The approximate cmc ranges for the surfactants tested are shown in Table 2.

TABLE 1. TRADE NAMES, FORMULAS, CHEMICAL TYPES, AND SUPPLIERS OF SURFACTANTS TESTED

Trade Name	Class/Formula	Nonionic	Anionic	Cationic	Amphoteric	Manufacturer
Triton X-100	Isooctyl phenyl polyethoxy ethanol	X				Rohm & Haas Company
Triton X-152	Blend of alkylarylpolyether alcohols and organic sulfonates		X			
Triton X-172	A blend as above		X			
Triton X-400 (82%)	Stearyl dimethyl benzyl ammonium chloride			X		
Triton QS-15	Oxyethylated sodium salt of amphoteric surfactant				X	
Tween 20	Polyoxyethylene sorbitan monolaurate	X				Atlas Chemical Industries, Inc.
Tween 40	Polyoxyethylene sorbitan monopalmitate	X				
Tween 60	Polyoxyethylene sorbitan monostearate	X				
Tween 80	Polyoxyethylene sorbitan monooleate	X				
Tergitol TMN (90%)	Trimethyl nonyl polyethylene glycol ether	X				Union Carbide Corporation
Tergitol 15-S-9	Polyethylene glycol ether of linear sec. alcohol	X				
Dow Corning XZ-8-3063	Silicone glycol copolymer	X				Dow Corning Corporation
Dow Corning 471 Fluid	Silicone glycol copolymer	X				
Pluronic L101	Ethylene oxide plus hydrophobic base of propylene oxide condensed with propylene glycol	X				Wyandotte Chemicals Corporation
Tetronic 901	Addition of propylene oxide to ethylenediamine, followed by addition of ethylene oxide	X				
Multifilm X-77 (80%)	Alkylarylpolyoxyethylene glycols and free fatty acids	X				Colloidal Products Corporation
Blendene	Terpene fatty acid salt complex		X			Glyco Chemicals, Inc.
Sodium lauryl sulfate	Sodium lauryl sulfate U.S.P.		X			Fisher Scientific Co.
Vatsol OT (70%)	Dioctyl ester of sodium sulfosuccinic acid		X			American Cyanamid Company
Alkaterge C	Substituted oxazoline			X		Commercial Solvents Corp.
Peregal ST	Polyvinylpyrrolidone			X		General Aniline & Film Corp.
Quaternary Ammonium Cpd. ADB 52734-R	Quaternary ammonium compound			X		California Research Corp.

TABLE 2. APPROXIMATE CRITICAL MICELLE CONCENTRATION (CMC) RANGES AND BIODEGRADABILITY OF SURFACTANTS TESTED

Surfactant	Cmc range, %	Biodegradability
Triton X-100	0.01-0.05	+
Triton X-152	0.01-0.05	-
Triton X-172	0.01-0.10	-
Triton X-400	0.01-0.10	-
Triton QS-15	0.10-1.00	-
Tween 20	0.01-0.05	-
Tween 40	0.05	-
Tween 60	0.01-0.05	-
Tween 80	0.01-0.05	-
Tergitol TMN	0.10-0.50	-
Tergitol 15-S-9	0.01-0.05	+
Dow Corning XZ-8-3063	0.05-0.10	-
Dow Corning 471 Fluid	0.05-0.10	-
Pluronic L101	0.01	-
Tetronic 901	0.01-0.05	-
Multifilm X-77	0.01-0.05	-
Blendene	0.10-0.50	+
Sodium lauryl sulfate U.S.P.	0.01	+
Vatsol OT	0.05-0.10	+
Alkaterge C	0.05-0.10	-
Peregal ST	1.00-5.00	+
Quaternary Ammonium Compound ADB	0.05	-

III. RESULTS

Mitotic inhibition and toxicity were induced by certain compounds in every ionogenic class except amphoteric (Table 3). However, because only one amphoteric compound was tested, generalizations cannot be made about this class of compounds. Only four of the 22 compounds tested showed no recognizable biological effect. One of these was the amphoteric Triton QS-15; others were the cationic Peregal ST and the nonionics Pluronic L101 and Tetronic 901.

TABLE 3. MITOTIC INDEX CHANGES AND TOXICITY CAUSED IN PISUM ROOTS BY SURFACTANTS AT 0.1%

Surfactant	Mitotic Index (% of control)	Toxicity
Triton X-100	18.7	+
Triton X-152	51.5	-
Triton X-172	12.2	+
Triton X-400	27.8	+
Triton QS-15	94.1	-
Tween 20	53.6	-
Tween 40	75.6	-
Tween 60	87.9	-
Tween 80	87.7	-
Tergitol TMN	34.6	+
Tergitol 15-S-9	15.0	+
Dow Corning XZ-8-3063	51.9	-
Dow Corning 471 Fluid	63.5	-
Pluronic L101	95.7	-
Tetronic 901	99.0	-
Multifilm X-77	28.9	-
Blendene	14.8	+
Sodium lauryl sulfate U.S.P.	15.2	+
Vatsol OT	10.1	+
Alkaterge C	44.5	+
Peregal ST	94.0	-
Quaternary Ammonium Compound ADB	10.0	+

All anionic surfactants tested were inhibitory to cell division, and several were also highly toxic. All cationic surfactants tested, with the exception of Peregal ST, inhibited mitosis initially and eventually resulted in the death of the primary root. The nonionic surfactants usually are considered the least reactive and thus the least biologically effective class of surfactants. Eight of the 12 nonionics that were tested inhibited mitosis; three of these were also toxic. Tween 60 and Tween 80 appeared to depress the mitotic rate slightly, although not appreciably. There appeared to be a trend toward increased mitotic inhibition by the lower members of the Tween series, which are derived from fatty acids with shorter chains than the higher members.

The silicone copolymers, Dow Corning XZ-8-3063 and Dow Corning 471 Fluid, which are very efficient in lowering the surface tension of aqueous solutions, caused a partial inhibition of mitosis but were not toxic.

IV. DISCUSSION

One type of inhibition of cell division was shown by Nethery and Wilson[17] to result from a blockage in the mitotic cycle prior to prophase; it may be recognized by changes in the mitotic index. A minimum in the mitotic index of the pea root meristem at 4 hours after the initiation of the treatment was a good index of pre-prophasic inhibition of mitotic activity. Mitotic disruption by exogenous chemicals may result from a simultaneous inhibition at several points of the mitotic cycle.[17,18] The extent to which each specific susceptible stage is affected depends on the particular chemical and dosage used. A complete cytological analysis at several intervals after treatment with each surfactant would provide detailed information on the several types of disturbances of the processes of cell division. However, all surfactants that showed effects at the cellular level at the concentrations tested in this study also induced pre-prophasic inhibition of mitosis. No attempt is made here to delineate the various other points of inhibition of mitosis or the chromosomal aberrations that may be induced by individual compounds, because none of these effects appears to be common to all surfactants tested. Neither does pre-prophasic inhibition indicate a biological disturbance that is due to surfactants as a class, or to specific chemical or physical properties. Because of the diversity of chemical structures among the surfactants tested, a common theory for the mode of action in inducing these disturbances cannot yet be formulated. Furthermore, the present state of knowledge does not provide evidence that such disturbances are the primary result of interactions between surfactant and plant tissue; alternatively, they may be a secondary effect caused by a primary biochemical or biophysical "lesion."

Some surfactants often thought to have no significant biological effects may inhibit mitosis and growth or prove toxic at levels that are commonly used to suspend or emulsify chemicals or to lower the surface tension of a solution. Two of the most efficient surface tension depressants tested, Dow Corning XZ-8-3063 and Dow Corning 471 Fluid, produced only a partial inhibition of mitosis and were not toxic. These findings appear to correspond with the argument by Jansen[12] that various biological effects of surfactants are not due entirely to lowered surface tension. Probably, chemical and physical forces resulting from the type and specific chemical structure of the surfactant are the determinants of biological activity. Many surfactants of widely differing chemical structures may be added to the already extensive variety of compounds known to inhibit cell division.

The toxicity shown by five of the six known biodegradable surfactants at levels of 0.1% or less warrants further study, because this type of surfactant is potentially important in eliminating problems of waste disposal and water pollution. Because the available information on the characteristics of many of the surfactants is very meager, it is possible that some of the other surfactants are also biodegradable. Further, there is no reason to assume that these biodegradable compounds (Table 2) are especially representative of such surfactants as a whole. However, one may speculate that the capability of undergoing biological degradation may render the surfactant potentially toxic to some biological systems. Such a toxic potential may be realized through the reactivity of the compound itself or through breakdown products that are more toxic than the parent compound.

When surfactants are used as research tools in an experimental system, it appears essential to examine first the effects, however slight, that the surfactants may have on the system. A basic understanding of the action of surfactants in biological systems will help in establishing a logical basis for their use as adjuvants in many facets of biological research.

V. SUMMARY

The effect of surface-active agents on mitosis was studied by applying 22 compounds, including representatives of the four major ionogenic classes, to a standardized pea root meristem test system.

Mitosis was inhibited by 16 surfactants at 0.1% v/v; the ionogenic type appeared to be unimportant. Two surfactants caused a slight depression in the mitotic index; the remaining four had no recognizable effect.

Several surfactants (nonionic, anionic, and cationic) were toxic at 0.1%. Of the six known biodegradable surfactants tested, five were toxic at 0.1%.

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13. ABSTRACT
Twenty-two ionic and nonionic surface-active agents were applied to a standardized pea root meristem test system. Mitosis was inhibited by 16 surfactants at concentrations of 0.1% v/v. Two surfactants caused a slight depression in the mitotic index; the remaining four had no recognizable effect. Several compounds were irreversibly toxic at levels of 0.1%. Five of the six known biodegradable surfactants tested were toxic.

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